

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
21 May 2004 (21.05.2004)

PCT

(10) International Publication Number  
**WO 2004/042050 A3**

(51) International Patent Classification<sup>7</sup>: **C12N 9/96**,  
11/00, 9/04, 9/02

(21) International Application Number:  
PCT/EP2003/012794

(22) International Filing Date:  
7 November 2003 (07.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
02079675.1 8 November 2002 (08.11.2002) EP

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*): AVANTIUM INTERNATIONAL B.V. [NL/NL]; Zekeringstraat 29, NL-1014 BV Amsterdam (NL).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): SMITH, Alan, Arthur [GB/NL]; Popelingstraat 10, NL-2011 ZL Haarlem (NL). WEGMAN, Margaretha, Anna [NL/NL]; Aziëlaan 254, NL-2622 JN Delft (NL).

(74) Agent: WITTOP KONING, T., H.; Exter Polak & Charlois B.V., P.O. Box 3241, NL-2280 GE Rijswijk (NL).

**Published:**

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:  
15 July 2004

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: IMPROVED CROSSLINKED ENZYME AGGREGATES

(57) Abstract: Described is a method for the preparation of crosslinked enzyme aggregates, comprising enzyme molecules being associated with an enzyme activity-enhancing agent, the enzyme activity-enhancing agent interacting with the said enzyme molecules, comprising the steps of: A - providing a plurality of enzyme molecules, B - aggregating the enzyme molecules in a liquid medium, by means of addition of a precipitating agent, C - crosslinking the aggregated enzyme molecules to one another by providing a crosslinking agent in the liquid medium, the method comprising, during or between steps A, B or C, the step of adding an enzyme activity-enhancing agent and allowing the enzyme molecules to become and to remain associated with the enzyme activity-enhancing agent. The resulting CLEA are advantageous due to increase in activity, their suitability for recycle, enhanced stability and low requirements for additional enzyme activity-enhancing agent. Further, crosslinked enzyme aggregates are described, comprising an enzyme activity-enhancing agent associated therewith.



WO 2004/042050 A3

## INTERNATIONAL SEARCH REPORT

PCT/JP 03/12794

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/96 C12N11/00 C12N9/04 C12N9/02

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>LOPEZ-SERRANO P ET AL: "Cross-linked enzyme aggregates with enhanced activity: Application to lipases."  BIOTECHNOLOGY LETTERS,  vol. 24, no. 16, August 2002 (2002-08),  pages 1379-1383, XP009003775  August, 2002  ISSN: 0141-5492  cited in the application  * page 1380, "CLEAs prepared in the presence of SDS", "CLEAs prepared in the presence of Triton X-100" *  * Figure 1 *  * page 1382, "Effect of additives" : first three paragraphs *  tables 1,2</p> <p style="text-align: center;">--- -/--</p>	1-15

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&amp;\* document member of the same patent family

Date of the actual completion of the international search

28 April 2004

Date of mailing of the international search report

06/05/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Mauhin, V

## INTERNATIONAL SEARCH REPORT

PCT/JP 03/12794

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 1 088 887 A (DSM NV) 4 April 2001 (2001-04-04) cited in the application ---	
A	WO 02/061067 A (VAN RANTWIJK FREDERIK ;CAO LINQIU (NL); UNIV DELFT TECH (NL); LOPE) 8 August 2002 (2002-08-08) ---	
A	DE 44 19 024 C (INST CHEMO BIOSENSORIK) 19 October 1995 (1995-10-19) -----	

# INTERNATIONAL SEARCH REPORT

PCT/EP 03/12794

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 1088887	A	04-04-2001	EP	1088887 A1	04-04-2001
WO 02061067	A	08-08-2002	NL	1017258 C2	02-08-2002
			CA	2436087 A1	08-08-2002
			EP	1360285 A1	12-11-2003
			WO	02061067 A1	08-08-2002
DE 4419024	C	19-10-1995	DE	4419024 C1	19-10-1995